Claims 24, 32 and 40 find support in the specification at page 5, lines 18-24. Newly added Claim 25 finds support in the specification at page 6 lines 6-8. Newly added Claims 26, 33 and 38 find support in the specification at page 8 lines 7-8 and page 9 lines 28-30. Newly added Claims 27, 34 and 38 find support in the specification at page 9 lines 29-31. Newly added Claims 28 and 35 find support in the specification at page 7 line 23 to page 8 line 6, and page 12 lines 23-24. Newly added Claims 29, 36, 37, 43 and 44 find support in original Claim 20 and the specification at page 5 lines 7-9. Newly added Claims 30 and 38 find support in original Claim 17 and the specification at page 17 lines 28-30. Newly added Claims 31 and 39 find support in original Claim 18 and the specification at page 5 lines 12-18. Newly added Claim 41 finds support in the specification at page 7 lines 23-29, and page 9 lines 25-27. Newly added Claim 42 finds support in the specification at page 12 lines 23-24.

Attached hereto is a marked up version of the changes made to the claims by the current amendment. The attached is captioned **"Version with Markings to Show Changes Made"**. No new matter has been added by these amendments and thus the entry thereof is respectfully requested.

Claim Rejections - 35 U.S.C. §103(a)

Claims 17-20 have been rejected under 35 U.S.C. §103(a) over Martin et al. (RNA, (1998), Vol.4, pages 226-230) in view of Cao et al. (Proceedings of the National Academy of Sciences, (USA), (1996), Vol.93, pages 11580-11585.) and further in view of Stratagene Catalog (1988, page 39), ("Stratagene"). The Office Action asserts that one of ordinary skill in the art would have been motivated to combine the above references to make the claimed invention. Specifically, the Office Action asserts that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to "combine and substitute the reagents and methods, wherein the prokaryotic poly(A) polymerase is a bacterial polymerase of Cao et al. into the composition of Martin et al."... and further to "combine a suitable container, non-radioactively labeled ribonucleotide and a prokaryotic poly(A) polymerase of Martin et al. in view of Cao et al. into a kit format as discussed by Stratagene."

According to MPEP §2142, an examiner must meet three basic criteria to establish a

prima facie case of obviousness: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference must teach or suggest all the claim limitations. The combination of Martin et al., Cao et al. and Stratagene do not meet all three of these criteria for the following reasons.

Martin et al. describes a method for end-labeling the 3'-end of RNA using a eukaryotic poly(A) polymerase and ATP-flourescein, ATP-biotin, ATP-digoxigenin, and UTP-digoxigenin, while Cao et al. simply teaches that bacterial (*Escherichia coli*) poly(A) polymerase has a potential use in mRNA polyadenylation. Stratagene merely teaches a motivation to combine reagents into a kit format.

The present application claims a kit for practicing methods of end-labeling ribonucleic acids with non-radioactive labels. A feature of the kit is that it includes at least a **prokaryotic** poly (A) polymerase and a non-radioactively labeled ribonucleotide.

It is respectfully submitted that the Examiner has not established a *prima facie* case of obviousness. The Office Action assumes that one of ordinary skill in the art would have been motivated to combine the bacterial polymerase of Cao et al. with the reagents and methods of Martin et al. However, the Applicant submits that the combination of the cited references would not have provided one of ordinary skill in the art with a **reasonable expectation of success**. As was understood by one of ordinary skill in the art at the time the invention was made, the use of prokaryotic [*Escherichia coli*] poly(A) polymerase to attach end-label ribonucleic acids with non-radioactive labels was believed to be impossible. For example, Rosemeyer et al. (U.S. Patent No. 5,573,913; issued November 12, 1996.) states:

> "The attachment of nucleotides to the 3' end of RNA molecules using...[*Escherichia coli*] poly(A) polymerase does...have considerable problems.... The efficient labelling of 3' ends of RNA molecules with [*Escherichia coli*] poly(A) polymerase is limited to the use of ATP and ATP derivatives since bases other than A are accepted much more poorly by the

> enzyme. Oligonucleotides have an extremely low efficiency as acceptor molecules. **The attachment of oligoribonucleotides to the 3' end of RNA molecules by** [*Escherichia coli*] **poly(A) polymerase is not known. It is not possible to attach non-radioactively labelled nucleotides**.... Therefore no process is known from the state of the art with which RNA molecules that are already present can be provided in a simple manner with one or several non-radioactive marker groups."
> [Column 1, line 58 through column 2, line 54.]

As shown by Rosemeyer et al., those of skill in the art did not believe it was possible to use a prokaryotic [*Escherichia coli*] poly(A) polymerase to end-label a ribonucleic acid with a non-radioactively labeled ribonucleotide. Thus, any attempt to do so by combining the cited references of Martin et al. and Cao et al. would not have been made with a reasonable expectation of success.

In addition, Cao et al., as mentioned above, only teaches that bacterial (*Escherichia coli*) poly(A) polymerase has a potential use in mRNA polyadenylation. There is no mention in Cao et al. of the polyadenylation of RNA using non-radioactively labeled ribonucleotides. In fact Cao et al. only describes the use of a radioactively labeled ribonucleotides, i.e. [^{3}H]ATP (Cao et al. pg. 11580). In contrast, the present application is directed to the end-labeling of ribonucleic acids with **non-radioactively labeled ribonucleotides**, of which Cao et al. is silent. Accordingly, the combined references of Martin et al. and Cao et al. fail to provide one of ordinary skill in the art with any reasonable expectation of success because the references fail to teach or suggest that a prokaryotic poly(A) polymerase could be used to end-label ribonucleic acids with non-radioactively labeled ribonucleotides.

Further, as is well known in the art, there are many important **differences between eukaryotic and prokaryotic poly(A) polymerases**. For example, as is described in Sarkar, (*Annu. Rev. Biochem* (1997) 66:173-97), prokaryotic poly(A) polymerases do not require an upstream consensus sequence such as the AAUAAA sequence, as is required by eukaryotic poly(A) polymerases (Sarkar, pp. 182 and 193); the poly(A) tracts of prokaryotic mRNAs are significantly shorter than those of eukaryotic mRNAs (Sarkar, pg. 175); and only a relatively

small fraction of mRNA molecules are polyadenylated in prokaryotes, "in contrast to the virtually quantitative polyadenylation of most eukaryotic mRNAs." (Sarkar, pg. 175). Accordingly, the substitution of the prokaryotic poly(A) polymerase of Cao et al. into the method of Martin et al. of non-radioactive end-labeling RNA using a eukaryotic poly(A) polymerase, would not have provided one of ordinary skill in the art with any reasonable expectation of success because of the significant differences between eukaryotic and prokaryotic poly(A) polymerases and the numerous other differences between eukaryotic and prokaryotic intracellular systems as was generally known in the art at the time the invention was made.

Thus the difficulties encountered by past researchers coupled with the significant differences between prokaryotic and eukaryotic poly(A) polymerases, would not have permitted one of ordinary skill in the art to combine the teachings of Martin et al. (fluorescent end-labeling of the 3'-end of RNA using eukaryotic poly(A) polymerase) and Cao et al. (mRNA polyadenylation using bacterial poly(A) polymerase) with any reasonable expectation of success. It was not until after the time of the Applicant's work, as reported in the present application, that one of ordinary skill in the art would have had a reasonable expectation of success in attaching non-radioactively (fluorescently) labeled ribonucleotides to the 3' end of ribonucleic acids with prokaryotic poly(A) (bacterial) polymerase.

As such, the claimed kits are not *prima facie* obvious to one having ordinary skill in the art at the time the invention was made because one of ordinary skill in the art would not have coupled the prokaryotic poly(A) polymerase with the non-radioactively labeled ribonucleotide in a kit for use in end-labeling ribonucleic acids, due to a lack of a reasonable expectation of success in using the two reagents together for any purpose.

As mentioned above, Stratagene merely teaches a motivation to combine reagents into a kit format. As such, the combined teachings of the references cited by the Examiner fail to provide one of skill in the art with a reasonable expectation of success in using prokaryotic poly (A) polymerases to end-label ribonucleic acids with non-radioactively labeled ribonucleotides. Therefore, the Examiner has failed to put forth a valid prima facie case of obviousness. As such, the Applicants respectfully request that this rejection of Claims 17-20

under 35 U.S.C. §103(a) be withdrawn.

Conclusion

The Applicant respectfully submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone Gordon Stewart at 650 485 2386. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-1078.

Respectfully submitted,

Date: 10. 31. 02 By: _____
Ted A. Chan
Registration No. 46,609

Date: 10. 31. 02 By: _____
Bret E. Field
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Enclosure

- Rosemeyer et al. (U.S. Patent No. 5,573,913).
- Sarkar, *Annu. Rev. Biochem* (1997) 66:173-97.

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Please amend Claim 19:

19. (Amended) The kit according to Claim 17, wherein said non-radioactively labeled ribonucleotide is [fluorescently] labeled with a directly detectable label.

Please cancel Claims 21-23.

Please add the following new claims:

--24. (New) The kit according to Claim 17, wherein said non-radioactively labeled ribonucleotide contains a modified nitrogenous base moiety covalently bonded to a non-radioactive label.

25. (New) The kit according to Claim 19, wherein said directly detectable label is a fluorophore.

26. (New) The kit according to Claim 25, wherein said fluorophore is a xanthenic fluorophore or polymethine fluorophore.

27. (New) The kit according to Claim 26, wherein said polymethine fluorophore is a cyanine fluorophore.

28. (New) The kit according to Claim 25, wherein said fluorophore is chosen from Pyrene, Coumarin, Diethylaminocoumarin, FAM, Fluorescein Chlorotriazinyl, Fluorescein, R110, Eosin, JOE, R6G, Tetramethylrhodamine, TAMRA, Lissamine, ROX, Napthofluorescein, Texas Red, FITC, HEX, Cy3, Cy5 and Cy7.

29. (New) The kit according to Claim 20, wherein said bacterial polymerase is chosen from Escherichia coli poly(A) polymerase 1, Escherichia coli poly(A) polymerase 2, Bacillus subtilis poly(A) polymerase 1, and Bacillus subtilis poly(A) polymerase 2.

30. (New) A kit for use in end-labeling ribonucleic acids with non-radioactively labeled ribonucleotides, said kit comprising:

a fluorescently labeled ribonucleotide; and

a prokaryotic poly (A) polymerase.

31. (New) The kit according to Claim 30, wherein said fluorescently labled ribonucleotide is a fluorescently labeled ATP analog, CTP analog, UTP analog or GTP analog.

32. (New) The kit according to Claim 30, wherein said fluorescently labeled ribonucleotide contains a modified nitrogenous base moiety covalently bonded to a fluorescent label.

33. (New) The kit according to Claim 30, wherein said fluorescently labeled ribonucleotide is labeled with a xanthenic fluorophore or polymethine fluorophore.

34. (New) The kit according to Claim 33, wherein said polymethine fluorophore is a cyanine fluorophore.

35. (New) The kit according to Claim 30, wherein said fluorescently labeled ribonucleotide is labeled with a fluorophore chosen from Pyrene, Coumarin, Diethylaminocoumarin, FAM, Fluorescein Chlorotriazinyl, Fluorescein, R110, Eosin, JOE, R6G, Tetramethylrhodamine, TAMRA, Lissamine, ROX, Napthofluorescein, Texas Red, FITC, HEX, Cy3, Cy5 and Cy7.

36. (New) The kit according to Claim 30, wherein said prokaryotic poly(A) polymerase is a bacterial polymerase.

37. (New) The kit according to Claim 36, wherein said bacterial polymerase is chosen from Escherichia coli poly(A) polymerase 1, Escherichia coli poly(A) polymerase 2, Bacillus subtilis poly(A) polymerase 1, and Bacillus subtilis poly(A) polymerase 2.

38. (New) A kit for use in end-labeling ribonucleic acids with non-radioactively labeled ribonucleotides, said kit comprising:

a fluorescently labeled ribonucleotide labeled with a xanthenic fluorophore or cyanine fluorophore; and

a prokaryotic poly (A) polymerase.

39. (New) The kit according to Claim 38, wherein said fluorescently labeled ribonucleotide is an ATP analog, CTP analog, UTP analog or GTP analog labeled with a Xanthenic fluorophore or Cyanine fluorophore.

40. (New) The kit according to Claim 38, wherein said fluorescently labeled ribonucleotide contains a modified nitrogenous base moiety covalently bonded to said fluorophore.

41. (New) The kit according to Claim 38, wherein said fluorescently labeled ribonucleotide is labeled with a Xanthenic fluorophore chosen from FAM, Fluorescein Chlorotriazinyl, Fluorescein, JOE, R110, R6G, Tetramethylrhodamine, TAMRA, Lissamine, ROX, FITC, and HEX.

42. (New) The kit according to Claim 38, wherein said fluorescently labeled ribonucleotide is labeled with a cyanine fluorophore chosen from Cy3, Cy5 and Cy7.

43. (New) The kit according to Claim 38, wherein said prokaryotic poly(A) polymerase is a bacterial polymerase.

44. (New) The kit according to Claim 43, wherein said bacterial polymerase is chosen from Escherichia coli poly(A) polymerase 1, Escherichia coli poly(A) polymerase 2, Bacillus subtilis poly(A) polymerase 1, and Bacillus subtilis poly(A) polymerase 2.--